IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lustig et al.	Group Art Unit: 1646
Serial No. ~~08/975,614~~ 09/163,913	Examiner: Pak, M.
Filed: November 21, 1997	Attorney Docket No. T97-012
For: *Nuclear Hormone Receptor Drug Screens*	

NDP 12/1/02

DECLARATION UNDER RULE 132

I, Keith R. Yamamoto, declare and state as follows:

1. I am a Professor of Biochemistry in the Department of Biochemistry and Biophysics at the University of California, San Francisco (UCSF) , and also serve as Director of the Biochemistry and Molecular Biology Program, and Chairman of the Department of Cellular and Molecular Pharmacology at UCSF. I have been a member of the American Academy of Arts and Sciences since 1989 and a member of the National Academy of Sciences since 1990. Over decades of research in cell biology, I have been presented numerous nationally recognized honors and awards, have served and continue to serve on numerous academic editorial boards and numerous federal government public advisory committees. I am a recognized expert in the field of cell biology and have authored hundreds of publications in this field. I serve as member of the scientific advisory board of Tularik, Inc., the assignee of this application.

2. Heery et al. (1997, Nature 387,733-36) describes three experiments: the first is an in vivo yeast-based two-hybrid experiment wherein a DNA-binding domain fusion protein comprising LXXLL motifs activated transcription through a ligand-binding domain of an estrogen receptor (Fig.1). The second experiment is a GST pull-down experiment wherein GST-ER fusion proteins pulled down in vitro translated ^{35}S-labeled natural-sequence SRC-1 proteins, but not otherwise identical mutant-sequence SRC-1 proteins wherein all four functional LXXLL motifs were disabled (Fig.3a). In this experiment, the natural sequence SRC-1 pull down was inhibited by uM concentrations of LXXLL peptides (Fig.3b). The third experiment showed that natural SRC-1 but not mutant SRC-1 increased activation of estrogen receptor in HeLa cells transiently transfected with a reporter plasmid (Fig.3c).

One skilled in the art would not construe Heery to suggest the feasibility of assaying direct, in vitro LXXLL peptide binding to purified receptor; in fact, to one skilled in the art, Heery suggests the opposite - that such an assay would not be feasible. First, Heery provides no data suggesting an LXXLL peptide can directly bind the receptor. Heery=s two-hybrid transcriptional activation is performed within yeast cells, the GST-pull down assay is performed in a crude cellular lysate, and the third, transient transfection experiment is cell-based. One skilled in the art would recognize that all of these experiments report both higher and lower order complex formation and that none of them implies

direct peptide-receptor binding.

Similarly, none of Heery=s data imply that an LXXLL peptide is sufficient to bind the receptor; in fact, they suggest the opposite. For example, one skilled in the art would recognize that Heery does not report any GST-pull down of an LXXLL peptide, nor any data wherein a single LXXLL motif was disrupted, but only wherein all four were disrupted. In fact, one skilled in the art would conclude that the author=s failure to provide such data suggests that single LXXLL-motif disruptions may not have worked. In fact, this negative inference is further compelled by the subsequent inhibition experiments, wherein the authors only report data wherein uM concentrations of peptide were required to inhibit pull down - orders of magnitude higher than the amount of the SRC-1 protein present (the disclosed in vitro transcriptional yield is on the order of a few nM). One skilled in the art would conclude that the disproportionately high concentration of peptide necessary to inhibit SRC-1 pull down suggests that LXXLL peptides do not provide sufficient receptor binding affinity to permit a direct, binding assay.

In short, none of Heery=s data suggest that the single peptides would be able to directly bind purified receptor proteins. Viewed through the eyes of one skilled in the art, Heery teaches away from an assay that relies on direct, in vitro, ligand-dependent LXXLL peptide binding to purified receptor, particularly one that requires lower, particularly sub-micromolar peptide concentrations.

3. In fact, when the project that led to this invention was first proposed over five years ago, neither I nor Steven McKnight, serving as scientific advisors, expected the ligand-dependent NHR binding assay to work with small peptide sensors as claimed. At the time, Tularik Inc. was seeking to set up a commercial assay for NHR ligands and we felt that existing assay formats, such as the gel-based coactivator dependent receptor ligand assay (Krey et al., 1997, Mol Endocrinol 11, 779-791), were not suitable for high-throughput applications. Dr. McKnight and I were well aware of publications, including the cited Heery et al., which had attempted to characterize NHR - coactivator binding requirements, and had identified coactivator regions necessary for binding. After carefully reviewing these publications, we concluded that the evidence provided by these papers did not suggest that small single LXXLL-motif peptides would be sufficient to bind receptor in a ligand-dependent manner sufficient to construct a binding assay as claimed. In fact, as noted above, we inferred the opposite B that Heery=s failure to provide any direct binding evidence and their disclosure that orders of magnitute higher concentrations of peptides were necessary to inhibit coactivator pull-down, suggest to us skilled in the art that such small peptides did not, and would not, provide sufficient binding specificity and affinity to construct a direct binding assay as claimed. Hence, at the project review meeting, both Dr. McKnight and I communicated our pessimistic assessment of the likelihood of succeeding with the proposed assay development project.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: August 29, 2002

Keith R. Yamamoto

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lustig et al.	Group Art Unit: 1646
Serial No. 08/975,614	Examiner: Pak, M.
Filed: November 21, 1997	Attorney Docket No. T97-012
For: *Nuclear Hormone Receptor Drug Screens*	

DECLARATION UNDER RULE 132

I, Jin-Long Chen, declare and state as follows:

1. I am a Director of Biology at Tularik Inc, the assignee of the above application. I am an expert in the field of cell biology, with particular expertise in nuclear hormone receptor biology. I am a coinventor of the above application. I hold a Ph.D. in Molecular Biology from the University of California at Berkeley.

2. Heery et al. (1997, Nature 387,733-36) describes three experiments: the first is an in vivo yeast-based two-hybrid experiment wherein a DNA-binding domain fusion protein comprising LXXLL motifs activated transcription through a ligand-binding domain of an estrogen receptor (Fig.1). The second experiment is a GST pull-down experiment wherein GST-ER fusion proteins pulled down in vitro translated ^{35}S-labeled natural-sequence SRC-1 proteins, but not otherwise identical mutant-sequence SRC-1 proteins wherein all four functional LXXLL motifs were disabled (Fig.3a). In this experiment, the natural sequence SRC-1 pull down was inhibited by uM concentrations of LXXLL peptides (Fig.3b). The third experiment showed that natural SRC-1 but not mutant SRC-1 increased activation of estrogen receptor in HeLa cells transiently transfected with a reporter plasmid (Fig.3c).

One skilled in the art would not construe Heery to suggest the feasibility of assaying direct, in vitro LXXLL peptide binding to purified receptor; in fact, to one skilled in the art, Heery suggests the opposite - that such an assay would not be feasible. First, Heery provides no data suggesting an LXXLL peptide can directly bind the receptor. Heery=s two-hybrid transcriptional activation is performed within yeast cells, the GST-pull down assay is performed in a crude cellular lysate, and the third, transient transfection experiment is cell-based. One skilled in the art would recognize that all of these experiments report both higher and lower order complex formation and that none of them implies direct peptide-receptor binding.

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receptor; in fact, they suggest the opposite. For example, one skilled in the art would recognize that Heery does not report any GST-pull down of an LXXLL peptide, nor any data wherein a single LXXLL motif was disrupted, but only wherein all four were disrupted. In fact, one skilled in the art would conclude that the author=s failure to provide such data suggests that single LXXLL-motif disruptions may not have worked. In fact, this negative inference is further compelled by the subsequent inhibition experiments, wherein the authors only report data wherein uM concentrations of peptide were required to inhibit pull down - orders of magnitude higher than the amount of the SRC-1 protein present (the disclosed in vitro transcriptional yield is on the order of a few nM). One skilled in the art would conclude that the disproportionately high concentration of peptide necessary to inhibit SRC-1 pull down suggests that LXXLL peptides do not provide sufficient receptor binding affinity to permit a direct, binding assay.

In fact, our own data showed that there is no necessary correlation between inhibitory activity and receptor binding. For example, SRC 1427-1440 is reportedly inhibitory in Heery=s coactivator - NHR binding inhibition assay (Fig.3a), yet we found the exact same peptide insufficient to directly bind three different receptors in our binding assay (Specification, p.6, lines 24-27).

In short, none of Heery=s data suggest that the single peptides would be able to directly bind purified receptor proteins. Viewed through the eyes of one skilled in the art, Heery teaches away from an assay that relies on direct, in vitro, ligand-dependent LXXLL peptide binding to purified receptor, particularly one that requires lower, particularly sub-micromolar peptide concentrations.

3. In fact, when the project that led to this invention was first proposed over five years ago, neither I nor Professors Keith Yamamoto and Steven McKnight, expected the ligand-dependent NHR binding assay to work with small peptide sensors as claimed. At the time, Tularik Inc. was seeking to set up a commercial assay for NHR ligands and we felt that existing assay formats, such as the gel-based coactivator dependent receptor ligand assay (Krey et al., 1997, Mol Endocrinol 11, 779-791), were not suitable for high-throughput applications. Drs. McKnight, Yamamoto and I were well aware of publications, including the cited Heery et al., which had attempted to characterize NHR - coactivator binding requirements, and had identified coactivator regions necessary for binding. After carefully reviewing these publications, we concluded that the evidence provided by these papers did not suggest that small single LXXLL-motif peptides would be sufficient to bind receptor in a ligand-dependent manner sufficient to construct a binding assay as claimed. In fact, as noted above, we inferred the opposite B that Heery=s failure to provide any direct binding evidence and their disclosure that orders of magnitute higher concentrations of peptides were necessary to inhibit coactivator pull-down, suggest to us skilled in the art that such small peptides did not, and would not, provide sufficient binding specificity and affinity to construct a direct binding assay as claimed. Hence, at the project review meeting, Drs. McKnight, Yamamoto and I communicated our pessimistic assessment of the likelihood of succeeding with the proposed assay development project.

4. Our patent application teaches that L_1-L_3 are independently selected from hydrophobic amino

acids, preferably leucine or isoleucine, more preferably leucine. See Specification, p.4, lines 26-28. The claimed methods require a peptide which provides direct, in vitro ligand-dependent binding to a nuclear hormone receptor. The Specification exemplifies the sensors and methods with a wide variety of suitable exemplary peptides with several receptors. Specification, p.5, line 23 - p.7, line 11. Though our exemplified list focused on our preferred embodiment wherein L_1- L_3 are leucine, we have in fact successfully practiced the claimed assay wherein L_1- L_3 are non-leucine hydrophobic amino acids, such as isoleucine and valine (see attachment e.g. TUK-1620-20, 1621-23, 1620-23).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: August 29, 2002

Jin-Long Chen

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα 25ng/uL 24,25 ex. 8uM ΔmP	LXRβ, 25ng/uL 24,25 ex. 8uM ΔmP	FXR, 35ng/uL cDCA, 80uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 28ng/uL BRL, 0.6uM ΔmP	PPARγ, 35ng/uL GW, 0.1uM ΔmP
SRC-1 632-640									
TUK-1385-60	R-	KLVQLLTTT	37	57.95	15	2.45	87.1	20.45	23.15
TUK-1305-66	R-	KLVQLLTTT	9.85	8.85	5.95	10.0	65.95	8.05	15.6
TUK-1387-60	R-G-	KLVQLLTTT	23.8	39.15	13.45	2.15	141.1	26.6	2.05
TUK-1387-62	R-G-	KLVQLLTTT	26.05	14.3	1.6	75.85	105.4	13.75	24.75
TUK-1387-70	R-G-	KLVQLLTTT	14.95	23.75	19.8	0	152.3	22.2	7.75
TUK-1387-72	R-G-	KLVQLLTTT	2.9	26	8.3	10.8	154.8	11.4	6.95
SRC-1 689-696									
TUK-1668-31	R-	ILHRLL	14.75	13.75	5.5	5.8	-3.65	12.55	6.95
TUK-1668-36	R-	ILHRLL	16.05	0.55	16.95	4.4	-0.95	12.4	6.55
SRC-1 689-696									
TUK-1371-58	R-	ILHRLLQE	70.25	137.7	41.25	84.4	108.7	41.5	90.6
TUK-1371-62	R-	ILHRLLQE	19.5	21	107.7	77.5	56	22.15	-2.05
TUK-1373-57	R-G-	ILHRLLQE	34.45	42.7	16.9	12.5	73.95	23.1	23
TUK-1373-63	R-G-	ILHRLLQE	27.05	70.35	12.5	13.75	98.6	17.05	0.75
SRC-1 746-756									
TUK-1474-52	R-	SQLLRYLLDKD	23.2	46.55	16.7	12.8	83.2	13.15	6.6
TUK-1474-61	R-	SQLLRYLLDKD	20.75	36.3	23.65	8.05	65.7	9.4	-1.7
SRC-1 747-756									
TUK-1473-70	R-	QLLRYLLDKD	27.35	133.05	101.55	14.45	101	17.15	25.5
TUK-1473-75	R-	QLLRYLLDKD	23.75	129.3	93.15	5.65	87.55	17.45	13.4
SRC-1 748-753									
TUK-1457-46	R-	LLRYLL	20.0	-35.4	0.15	-9.25	-8.05	-0.2	-3.95
TUK-1457-49	R-	LLRYLL	3.45	-37.65	-6.1	1.25	-1.2	13.65	-6.95
TUK-1670-44	R-	LLRYLL	19.55	-6.2	8.75	5.60	-0.35	3.9	0.6
TUK-1670-46	R-	LLRYLL	8.39	-14.15	-3	16.95	0.75	5.05	1.7
SRC-1 748-754									
TUK-1459-18	R-	LLRYLLD	16.35	23.2	37.35	0.2	33.55	5.5	-5.65
TUK-1453-22	R-	LLRYLLD	17.95	-5.40	-17.7	6.35	33	5.2	3.0
SRC-1 749-759									
TUK-1391-73	R-	LLRYLLDK	309.9	75.3	99.65	72.65	37.9	43.85	37.7
TUK-1391-76	R-	LLRYLLDK	117.35	7.2	23.15	83.05	45	37.65	12.65
TUK-1393-58	R-G-	LLRYLLDK	42.75	69	20.5	5.1	60.0	21.15	11
TUK-1393-61	R-G-	LLRYLLDK	38.8	51.15	22.35	3.2	45.1	13	13
SRC-1 748-756									
TUK-1472-61	R-	LLRYLLDKD	123.05	75.95	107.7	6.25	77.15	46.5	35
TUK-1472-64	R-	LLRYLLDKD	53.4	51.75	99.1	12.15	66.9	19.7	25.35
SRC-1 749-753									
TUK-1459-43	R-	LRYLL	9.55	17.4	7.05	79.25	8.1	18.2	8.55
TUK-1459-46	R-	LRYLL	14.45	5.35	3.5	1.75	-1.8	20.9	4.4
SRC-1 749-754									
TUK-1455-40	R-	LRYLLD	0.2	74.15	57.9	0.5	11.25	27.4	37.1
TUK-1455-43	R-	LRYLLD	8.45	11.1	5.65	2.5	6.3	10.6	4.25
SRC-1 1427-1440									
TUK-1399-71	R-G-	FQAQQKSLLQQLLT	5.7	16.85	11.2	6.7	10.85	10.1	10.15
SRC-1 1434-1441									
TUK-1381-67	R-	LLQQLLTE	7.95	27.1	9	17.7	42.7	10.1	14.15
TUK-1381-69	R-	LLQQLLTE	38.8	83.1	47	69.4	48.6	11.65	27.65
TUK-1383-55	R-G-	LLQQLLTE	1.95	51.15	22.15	73.35	52	4	6.05
TUK-1383-61	R-G-	LLQQLLTE	15.4	30.3	10.95	6.9	66.4	12.05	8.05
RIP-140 132-139									
TUK-1631-27	R-	LLASLLQS	59.25	62.35	35.3	7.1	100.75	64.25	160.9
TUK-1631-29	R-	LLASLLQS	17.35	48.15	15.35	3.85	59.05	51.5	23.45
TUK-1631-30	R-	LLASLLQS	21.8	31.85	10.95	3.1	37.35	36.35	36.75
RIP-140 184-191									
TUK-1616-31	R-	HLKTLLKK	16.15	4.35	3.15	19.35	25.3	30.7	17.95
TUK-1616-35	R-	HLKTLLKK	2	9.35	3.2	-2.25	16.05	34.1	19.9
RIP-140 366-373									
TUK-1632-38	R-	QLALLLSS	9.05	16.5	12.5	10.1	19.4	17.35	18.35
RIP-140 379-386									

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10nM/uL 9cRA, 0.1uM ΔmP	LXRα, 25ng/uL 24,25 ex, 3uM ΔmP	LXRβ, 25ng/uL 24,25 ex, 3uM ΔmP	FXR, 25ng/uL cDCA, 20uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.2uM ΔmP	PPARγ, 25ng/uL GW, 0.1uM ΔmP
TUK-1633-31	R-	LLLPLLKD	116.2	135.35	99.3	13.2	142.5	84	94.2
TUK-1633-32	R-	LLLRLLKE	42.55	46.25	31.3	84.95	85.55	22.2	37.3
RIP-140 498-506									
TUK-1375-47	S-	VTLLQLLLQ	31.05	69.2	38.85	13.95	72.25	1.2	113.35
TUK-1375-51	R-	VTLLQLLLQ	44.9	79.45	-1.55	6.7	41.5	4.1	105
TUK-1377-72	R-G-	VTLLQLLLQ	38	58.45	8.15	17.7	67.8	7.95	9.75
TUK-1433-69			26.65	60.55	22	11.15	85.95	35.1	50.55
RIP-140 712-719									
TUK-1618-65	R-	VLQLLLGN	12.5	65.6	-1.3	14.95	9.55	12	7.6
TUK-1618-69	R-	VLQLLLGR	11.05	20.1	10.45	7.6	17.95	15.05	18.35
RIP-140 818-825									
TUK-1644-45	R-	LLSRLLRQ	99.75	43.1	23.35	89.15	32.9	16.65	18.4
TUK-1644-49	R-	LDSRLLRQ	10.9	8.7	7.8	13.85	38.8	10.45	13.95
RIP-140 935-942									
TUK-1608-43	R-	VLRQLLLP	38.05	18.15	21.2	9.8	35.6	30.1	18.65
RIP140									
TUK-1634-47	R-	VLRNLLNN	8.75	6.25	7.6	2.35	10.45	8.85	-5.05
CBP 68-									
TUK-1613-24	R-	QLSQLLRQ	8.7	33.1	2.95	-1.95	7.6	18.05	10.95
TUK-1615-26	R-	QLSQLLRQ	2.45	29.75	7.8	10	12.3	17.1	3.95
TUK-1615-27	R-	QLSQLLRQ	8.35	5.9	4.6	-0.25	6	12.85	18.45
CBP 356-									
TUK-1637-30	R-	QLVLLLRA	10.6	27.6	17.1	9	19.6	12.3	11.95
TUK-1637-42	R-	QLVLLLRA	3.85	13.15	-8.1	10.35	17.15	21	2.35
p120 339-346									
TUK-1629-37	R-	LLSELLNK	44	54.5	15.25	78.9	37.55	6.1	15.75
TUK-1629-39	R-	LDSELLNK	16.2	10.6	-3.5	26.55	24.8	7.7	12.15
p120 308-315									
TUK-1629-33	R-	TLSRLLRA	17.8	74.1	24.95	68.25	26.1	13.1	7.1
TUK-1629-36	R-	TLSRLLRA	4.85	21.1	4.25	17.3	15.45	23.35	-2.2
TRIP2 23-									
TUK-1642-36	R-	MSMNLLRD	36.05	72	34.4	9.6	51.95	32.65	30.1
TUK-1642-39	R-	MSMSLLRD	11.3	42.55	22.65	8.15	17.5	17.1	8.5
TRIP4 36-									
TUK-1643-35	R-	RLAVLLPQ	13.75	0.05	3	10.7	4.6	9.2	-2.05
TUK-1643-37	R-	RLAVLLPQ	2.4	6.55	-1.95	6.15	-1.2	13.65	8.05
TRIP5 26-									
TUK-1640-40	R-	RLSELLEV	18.95	51.95	8.15	14.9	30.1	12.35	12.35
TUK-1645-42	R-	RLSELLEV	8.7	14.95	5.1	80.15	14.75	30.25	12.55
TRIP8 36-									
TUK-1641-39	R-	TLNELLTT	6.9	13.35	12.25	6.3	32.8	21.1	11.5
TRIP9 73-									
TUK-1634-55	R-	RLDFLLGF	0.7	36.8	-17.3	8.3	-13.05	1.2	-1.85
TUK-1634-57	R-	RLDFLLGF	-36.85	-45.3	-59.4	-2	-23.75	-16.4	-21.05
TRIP9 356-									
TUK-1636-34	R-	VLRLLLRA	19.15	24.75	23.15	10.95	31.65	17.05	9.9
TUK-1636-59	R-	VLRLLLRA	9.2	24.05	-6.75	-0.8	16.5	7.9	2.5
TRIP9 288-									
TUK-1639-43	R-	YVAPLLRA	83.45	19.9	8.9	78.65	73.6	10.05	9.45
TIF1 722-									
TUK-1638-48	R-	RLTSLLLN	108.6	73.3	15.75	19.1	137.25	17.65	21.9
TUK-1638-50	R-	YLTSLLAN	38.6	11.15	2.38	5.15	89.3	11.4	4.35
TIF2 649-									
TUK-1635-36	R-	KLKQLLTT	55.05	88.85	40.9	7.05	144.1	38.6	33.55
TUK-1632-39	R-	KLKQLLTT	13.5	37.2	13	13.3	46.95	20.7	24.9
TUK-1635-40	R-	KLKQLLTT	9.35	30.25	7.25	13.15	87.6	7.85	13.55

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 1ong/uL 9cRA, 0.1uM ΔmP	LXRα, 25ng/uL 24,25 ex, 30uM ΔmP	LXRβ, 25ng/uL 24,25 cx, 30uM ΔmP	FXR, 25ng/uL cDCA, 50uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.6uM ΔmP	PPARγ, 25ng/uL GW, 0.1uM ΔmP
TIF3 37-									
TUK-1640-37	R-	TLRELLE	110.3	72.15	26.45	77.6	91.5	96.95	62.35
TUK-1640-40	R-	TLRELLE	116.2	61	19.05	93.65	95.25	49.75	64.05
TUK-1640-41	R-	TLRELLW	34.05	11.9	13.35	4.25	35.7	31.55	14.7
Non-co-activator									
TUK-1637-49	R-	ILRELLQE	120.05	132.5	20.05	80.9	92.7	96.75	82.4
TUK-1637-52	R-	ILRELLQE	69.25	50.05	18.7	6.65	91.25	97.65	61.45
TUK-1559-60	R-	ILRELLQE	186.75	72.4	28.45	13.55	108.85	89.5	82.4
TUK-1559-55	R-	ILRELLQE	147.05	97	36.35	7.6	96.05	99.05	55.75
TUK-1559-58	R-	ILRELLQE	73.25	66.05	44.6	8.2	145.9	86.6	86.7
TUK-1560-21	R-	ILRELLQE	132.25	81.8	37.15	71.9	106.7	86.65	80.65
TUK-1560-36	R-	ILRELLQE	232.6	142.9	93.25	77.6	149.8	105.8	130.4
TUK-1560-52	R-	ILRELLQE	255.6	142.6	86.95	80.65	141.9	107.7	148.2
TUK-1560-55	R-	ILRELLQE	103.35	72.9	20.15	6.65	91.95	86.7	91
TUK-1562-37	R-	ILRELLQE	31.95	45.55	17.3	8.35	32.3	21.85	14.95
TUK-1562-53	R-	ILRELLQE	9.55	7.35	4.1	7.25	14.85	9.3	8.3
TUK-1563-37	R-	ILRELLQE	28.2	52.75	16.1	13.5	66.15	17.85	16.75
TUK-1563-39	R-	ILRELLQE	28.05	7.55	11.9	12.45	23.15	8.1	6.75
TUK-1564-38	R-	ILRELLQE	30.2	91.75	10.55	15.9	67.95	21.7	20.85
TUK-1564-40	R-	ILRELLQE	13.15	10.15	-6.4	5.55	17.85	5.3	18.9
TUK-1565-60	R-	ILRELLQE	9.95	10.95	1.45	7.8	11.35	14.35	8.4
TUK-1565-42	R-	ILRELLQE	6.7	3.9	13.55	11.45	6.9	9.15	8.35
TUK-1568-39	R-	ILRELLQE	24.25	38.5	10.8	3.95	30.75	9.55	11.7
TUK-1569-41	R-	ILRELLQE	15	30.95	2.65	18.4	21.3	13.75	7.35
TUK-1569-37	R-	ILRELLQE	13.85	5.6	17.25	4.65	9.35	6.75	11.65
TUK-1569-39	R-	ILRELLQE	14.6	12.8	-1.1	6.85	3.55	12.1	4.95
TUK-1570-39	R-	ILRELLQE	53.85	124.95	43.6	12.65	60.6	23.05	34
TUK-1570-41	R-	ILRELLQE	29.4	24.55	7.65	1.3	21.05	21.25	9.7
TUK-1571-40	R-	ILRELLQE	122.65	121.6	30.4	84.15	152.7	45.5	55.45
TUK-1573-59	R-	ILRELLQE	85.85	52.05	29.1	75.85	81.5	24.35	17.8
TUK-1575-38	R-	ILRELLQE	-0.6	134.15	122.6	10.4	42.7	33.8	26.3
TUK-1575-37	R-	ILRELLQE	17.5	46.6	30.95	19.3	25.35	16.65	12.35
TUK-1576-45	R-	ILRELLQE	55	135.65	50.55	16.95	87.35	15.39	26.05
TUK-1576-47	R-	ILRELLQE	14.35	28.65	12	30.6	32.4	7.8	6.75
TUK-1577-43	R-	ILRELLQD	74.9	132.45	51.65	13.65	102.4	34.95	39.5
TUK-1577-45	R-	ILRELLQD	28.55	29.8	25.65	80.05	51.7	22.8	-0.8
TUK-1578-39	R-	ILRELLQD	63.1	148.9	52.65	30.7	73.7	28.55	24.45
TUK-1578-41	R-	ILRELLQD	22.75	32.9	17.45	14.9	20.05	14.4	3.65
TUK-1579-39	R-	ILRELLQD	20.1	33.3	9.7	18.15	49.35	18.35	18.2
TUK-1580-41	R-	ILRELLEE	25.25	82.15	91.55	3.45	56.6	7.85	14.65
TUK-1580-42	R-	ILRELLEE	21.25	36.4	12.75	17.25	37.2	10.4	2.8
TUK-1581-43	R-	ILRELLEE	34.95	20.1	9.65	7.95	65.55	29.45	9.05
TUK-1582-38	R-	ILRELLEE	34.3	16.7	-0.85	11.5	56.3	27.9	5.95
TUK-1586-49	R-	ILRELLEK	32.3	46.45	51.55	7.75	22.25	11.75	-0.9
TUK-1586-50	R-	ILRELLEK	16.05	49.4	11.1	13.9	33.1	91.6	8.35
TUK-1587-40	R-	ILRELLEE	48.4	38.45	39.8	7.4	66.45	23.75	28.05
TUK-1587-41	R-	ILRELLEE	32.75	23.3	30.5	8.15	57.75	20.25	18.85
TUK-1588-45	R-	ILRELLEE	40.9	62.2	50.35	12.25	30.9	16.15	6.5
TUK-1588-48	R-	ILRELLEE	-8.15	21.9	-1.35	92.4	47.25	30	12.65
TUK-1589-40	R-	ILRELLEE	46.3	89.1	21.05	76.25	62.1	14.55	17.95
TUK-1589-42	R-	ILRELLEE	12.95	19	9.7	11.35	23.2	10.35	9.5
TUK-1594-42	R-	ILRELLEE	48.35	78.15	35.1	7.05	41.35	7.55	10.8
TUK-1594-46	R-	ILRELLEE	16.85	16.1	10.75	10.35	26.2	12.6	12.25
TUK-1595-40	R-	ILRELLEE	26.05	17.75	1.2	5.1	18.2	9.55	15.8
TUK-1597-38	R-	ILRELLEE	17.65	5.85	4.25	-8.4	8.3	13.3	6.4
TUK-1599-47	R-	ILRELLEE	100.9	44	23.45	8.05	81.55	23.4	24.25
TUK-1601-46	R-	ILRELLEE	188	150.8	51.45	19.3	109.35	75.1	67.8
TUK-1601-49	R-	ILRELLEE	96.45	44.8	28.6	3.05	107.7	36.7	79.15
TUK-1603-51	R-	ILRELLEE	167.15	78.3	44.3	8.4	102.8	47.75	47.5
TUK-1606-44	R-	ILRELLEE	193.6	56.3	24.7	76.45	109.35	31.3	30.05
TUK-1607-45	R-	ILRELLEE	89	40.55	21.6	13	48.5	20.75	36.95
TUK-1607-45	R-	ILRELLEE	89	40.55	11.6	13	48.5	20.75	26.95
TUK-1610-42	R-	ILRELLEE	55.75	25.35	6.8	7.75	29.5	11.75	-3.75
TUK-1611-36	R-	ILRELLEE	330.85	136.35	116.4	76.05	155.4	118.4	108.5
TUK-1611-38	R-	ILRELLEE	146.3	36.25	33.45	64.95	95.95	37.45	47.85
TUK-1612-40	R-	ILRELLEE	90.65	69.35	19.35	3.9	51.05	8.35	15.4
TUK-1612-42	R-	ILRELLEE	84.95	62.85	21.7	8.15	80	20.95	15.6
TUK-1613-38	R-	ILRELLEE	152.75	46.65	18.15	9.7	58.5	31.6	37.7
TUK-1620-20	R-	ILRELVLQE	131.65	77.55	34.25	8.45	142.7	67.9	79.35
TUK-1620-23	R-	ILRELVLQE	130.7	50.95	8.15	7.15	84.7	21.7	24.6
TUK-1621-23	R-	ILRELLQE	234.6	61.95	16	5.8	103.15	18.9	21.8
TUK-1621-25	R-	ILRELLQE	130	29.85	-3.85	9.3	96.9	38.65	7
TUK-1622-21	R-	ILRELLQE	187.65	150.15	54.55	10.25	153.7	80.15	146.8
TUK-1622-29	R-	ILRELLQE	68.9	59.65	23.15	16.3	69.05	63.9	30.2
TUK-1624-21	R-	ILRELLLQE	191.65	103	56.95	11.05	153.6	60.6	55.45

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands			RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα 25ng/uL 24,25 ex, 3uM ΔmP	LXRβ, 25ng/uL 24,25 ex, 3uM ΔmP	FXR, 20ng/uL CDCA, 80uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.6uM ΔmP	PPARγ, 30ng/uL GW, 0.1uM ΔmP
TUK-1624-22	R-	ILLSKLLLQE	132.45	69.05	32.45	12.9	87.45	38.3	17.55
TUK-1624-23	R-	IaLRKLLQE	122.1	64.4	14.7	1.15	73.4	17.35	8.6
TUK-1625-21	R-	ILRciLLQE	274.5	88.25	40.5	11.4	109.75	91.9	113.4
TUK-1625-23	R-	ILRciLLQE	219.65	79.8	27.8	6.6	91.45	17	26.9
TUK-1626-45	R-	ILRRciLLLQE	279.4	139.6	37	27.2	144.8	100.55	143.25
TUK-1626-48	R-	ILRHciLLLQE	176.65	67.5	12.45	1.75	93.8	30.8	37.95
TUK-1627-45	R-	ILHciHciLLQE	139.3	89.8	48	10.95	141.2	67.3	73.55
TUK-1627-46	R-	ILHciHciLLQE	58.05	23.95	7.35	7.6	110	18	31.7
TUK-1630-19	R-	ILRKLLQD	183.1	84.3	95.75	14.35	106.35	79	113.3
TUK-1630-22	R-	ILRKLLQN	111.9	63.6	30.75	6.95	80.4	24.05	33.55
TUK-1646-21	R-	ILRKLLQci	141.15	56.25	35.45	14.35	75.65	60.05	65.3
TUK-1646-24	R-	ILRKLLQci	160.75	52.05	34.2	90.6	66.75	45.05	29.4
TUK-1647-22	R-	ILRKLLQHci	249.3	76.3	35.95	79.9	85.15	88.85	83.75
TUK-1647-25	R-	ILRKLLQHci	129.1	35.8	4.63	17.7	46.3	28.2	25.65
TUK-1648-27	R-	ILRKLLciE	246.5	131	117.6	26.4	107.3	123.9	157.2
TUK-1648-30	R-	ILRKLLciE	122.2	60.7	61.2	12.7	100.3	37.95	95.35
TUK-1649-29	R-	ILRKLLHciE	165.9	90.25	43.45	76.65	105.6	58.5	51
TUK-1649-30	R-	ILRKLLHciE	137.4	83.8	35.5	77.9	100.25	40.5	42.05
TUK-1650-29	R-	ILRKLLHciHci	104.55	35.3	4.85	5.9	43.5	38.45	20.05
TUK-1651-36	R-	ILRKLciHci	252.5	75.15	56.65	27.4	78.05	89.3	137.75
TUK-1651-39	R-	ILRKLciHci	163.35	42.6	20.55	10.45	66.55	29.3	29.7
TUK-1652-36	R-	ILRKLLcici	186.4	73.3	43.7	12.45	82.9	81.85	134.9
TUK-1652-37	R-	ILRKLLcici	131.55	64.25	16.7	76.3	63.4	19.8	26.9
TUK-1652-35	R-	ILRKLLHciHci	155.76	60.65	51.95	81.95	38	80.35	75.4
TUK-1653-37	R-	ILRKLLHciHci	126.3	36.1	14.85	16.3	54	35.6	45.4
TUK-1654-27	R-	ILRKLALQE	53.35	72.3	36.75	21.7	47.95	18.45	94.15
TUK-1654-29	R-	ILRKLALQE	17.05	23.05	-15.8	13.65	20.75	8.35	17.95
TUK-1655-37	R-	ILRKALQE	97.75	85.8	111.2	81.95	107	119.45	156.3
TUK-1655-38	R-	ILRKALQE	52.65	84.9	54.9	8.3	51.7	85.05	83.2
TUK-1656-37	R-	IALRKLLQE	81.9	69.2	49.65	5.3	63.75	31.6	28.1
TUK-1656-30	R-	FALRKLLQE	56.8	54.75	47.9	13.8	78.1	54.8	63.53
TUK-1657-36	R-	BLLSKLLQD	362.3	66.95	33.7	78.9	123.3	103.25	153.2
TUK-1657-38	R-	bLLSKLLQE	172.75	50.9	2.4	9.5	78	37.8	37.5
TUK-1750-40	R-	ILRKLLQEEbiot	176.2	47.15	24	11.7	97.3	42.6	63.35
TUK-1829-39	R-	ILRKLLDGbiot	40.4	56.55	84.9	17.7	59.2	26.65	17.2
TUK-1829-50	R-	ILHKLLHGbiot	35.33	31.95	14.85	13.45	30.65	31.2	17.75
TUK-1831-63	R-	ILRKLLQEbiot	31.35	29.5	2.6	4.1	37.7	14.7	19.3
TUK-1833-56	R-	ILRKLLEEbiot	32.53	83.85	61.95	13.15	53.45	15.75	-0.6
TUK-1833-58	R-	ILRKLLEEbiot	25.45	59.15	11.35	11.53	35.95	13.95	11.35
TUK-1835-39	R-	LLHKLLHGbiot	19.55	39.2	20.9	-3.25	47.9	33.8	28.6
TUK-1837-36	R-	LLLHLLEGbiot	28.75	56.2	23.65	13.3	93.6	32.3	11.45
TUK-1839-39	R-	ILRSLLLEDbiot	94.2	47.7	18.5	6.05	34.1	31.75	38.2
TUK-1841-38	R-	ILRKLLKEbiot	19.25	49.55	21.9	80.95	45.05	10.3	10.35
TUK-1843-40	R-	ILHciRLLGDbiot	75.7	57.75	6.8	3.75	147.4	39.85	34.05
TUK-1849-48	R-	ILRK-norleuLQEbiot	198.35	59.8	33	16.1	91.9	35.6	35.9
TUK-1851-47	R-	ILRKL-norleuQEbiot	63.4	38	21.5	13.13	31.2	6.1	4.45
STAT									
TUK-1617-45	R-	CACYVPVQDLYC	111.2	52.35	24.85	13.8	65.75	48	51.2
TUK-1617-48	R-	CAGYVPVQDLYC	18.9	-17.2	-7.95	6.8	5.6	6.1	10.45
YAMAMOTO									
TUK-1675-27		R-KHKILHRLLQDSSPVDLAK	19.75	18	17.3	-5.1	34.4	13.75	12.85
TUK-1679-29		R-KHKILHRLLQDSSPVDLAK	20.75	24.45	10.3	8.6	40.65	20.65	11.25
TUK-1679-54	R-	KHKILHRFLQDSSS	5	1.6	6.75	3.4	8.2	8.95	11.45
TUK-1679-59	R-	KHKILHRFLQDSSS	-1.7	10	8.55	10.4	0.3	19.25	-3.3
TUK-1681-34	R-	KHKILHRLFQDSSS	0.1	7.25	4.75	-1.65	0.35	2.8	10.35
TUK-1683-32	R-	KHKILHRLLQDSSS	12.35	2.95	11.75	8.65	26.45	21.05	5.95
TUK-1685-31	R-	KHKAAHRLLQDSSS	1.65	-22.55	-3.35	6.08	2.15	14.85	11.4
TUK-1685-34	R-	KHKAAHRLLQDSSS	11.85	4.65	1.5	5.5	5.9	20.3	14.05
TUK-1689-40	R-	KHKILAALLQDSSS	15.85	30.45	0.9	3.65	27	16.2	15.95
TUK-1689-42	R-	KHKILAALLQDSSS	14.35	23.55	17.05	8.05	65.5	18.9	-3.33
TUK-1691-32	R-	KHKILHRAAQDSSS	13.95	7.15	14.55	13.1	1	14.6	7.6
TUK-1691-19	R-	KHKILHRAAQDSSS	5.75	7.1	6.25	-4.75	10.35	9.95	9.85
TUK-1661-47	R-	GPEQILHRLLGIDE	-18.3	2.45	5.35	-6.55	5.45	11.25	6.55
TUK-1669-25	R-	GPEQLLRHDIGIDE	-0.3	2.9	2.5	12.7	29.9	8.15	9.96
TUK-1663-29	R-	GPEQLLRKLIGIDE	13.6	17.55	2.15	5.85	28.3	7.35	3.5
TUK-1665-41	R-	GPEQILTHLIGIDE	10.35	5.6	7.5	22.7	11.6	19.8	9.95
TUK-1665-47	R-	GPEQILTDLIGIDE	10.65	11.4	10.45	14.1	7.65	15.35	7.45
Perini									
TUK-1752-50	R-	LHKDLLDK	8.65	13.05	13.1	112.95	19.5	15.1	9.7
TUK-1752-61	R-	LHRKLLDK	2.3	2.35	11.5	4.65	7.9	9.55	2.7
TUK-1753-34	R-	LMRKLLDK	30.8	34.15	24.45	4.83	3.8	12.35	10.15
TUK-1753-37	R-	LMRKLLDK	6.7	31.5	13.1	10.85	13.95	13.75	13.6
TUK-1754-38	R-	LVRCYHDK	7.1	17.8	11.35	7.3	5.55	17.1	5.5

Peptide Activity in FP Assay

Receptors / Peptides \ Ligands		RXR, 10ng/uL 9cRA, 0.1uM ΔmP	LXRα, 25ng/uL 24,25 ex, 3uM ΔmP	LXRβ, 20ng/uL 24,25 ex, 8uM ΔmP	FXR, 15ng/uL cDCA, 20uM ΔmP	ERβ, 25ng/uL b-Estr., 0.1uM ΔmP	PPARγ, 25ng/uL BRL, 0.5uM ΔmP	PPARγ, 25ng/uL GW, 0.1uM ΔmP
TUK-1754-41	R- LVREYNDK	8.55	13.68	7.35	5.2	10.15	11.2	3.8
R-con								
TUK-1676-40	R- ELDNLLQQ	8.05	9.65	9.7	0.35	8.3	19	11.05
TUK-1676-44	R- EDDNLLQQ	6.05	11.35	3.2	4.55	3.25	20.05	10.45
TUK-1785-49	R- ELITLAHEQQITTQQWAR	1.4	2.2	17.3	70.75	-6.25	6.8	7.7
TUK-1785-53	R- ELITLAHEQQITTQQWAR	30.45	11.25	10.65	77.65	0.45	10.85	4.05
TUK-1787-28	R- GSITQQTPA	20.65	11.85	8.5	11.15	8.85	10.5	1.25
TUK-1787-33	R- GSITQQTPA	19.6	13.95	7.2	11.75	14.85	8.6	13.35
TUK-1789-24	R- EQHVKSIEQ	11.95	15.8	4.7	11.8	5.65	13.25	8.1
TUK-1789-28	R- KQHVKSIEQ	7.8	9.4	4.25	9.55	9.6	0.1	14.45
TUK-1793-50	R- [illegible]	13.15	-0.9	3.65	1.65	6.75	14.05	14.05
[illegible]								
TUK-1671-16	R- KLLQLLTC	27.55	38.6	5.95	11.55	145.1	37.35	39.95
TUK-1671-26	R- KLLQLLTC	25.25	30.3	17.35	21.6	148.6	23.3	19.5
TUK-1673-34	R- ILHKLLQH	110.3	137.6	45.1	-6.6	92.7	48.45	45.2
TUK-1672-37	R- ILHKLLQH	42.15	25.15	10.2	7.75	33.3	13	16.85
TUK-1673-40	R- LLRYLLDR	95.65	40.75	-8.15	31.5	47.3	26.2	33.25